

O
MAY 09 2003
PATENT & TRADEMARK OFFICE

TITLE: METHOD AND APPARATUS FOR
AUTOMATICALLY REMOVING VECTOR UNIT IN
DNA BASE SEQUENCE
INVENTORS: Kensaku IMAI, et al.
SERIAL NO.: 09/785,269
DOCKET NO.: 826.1335C

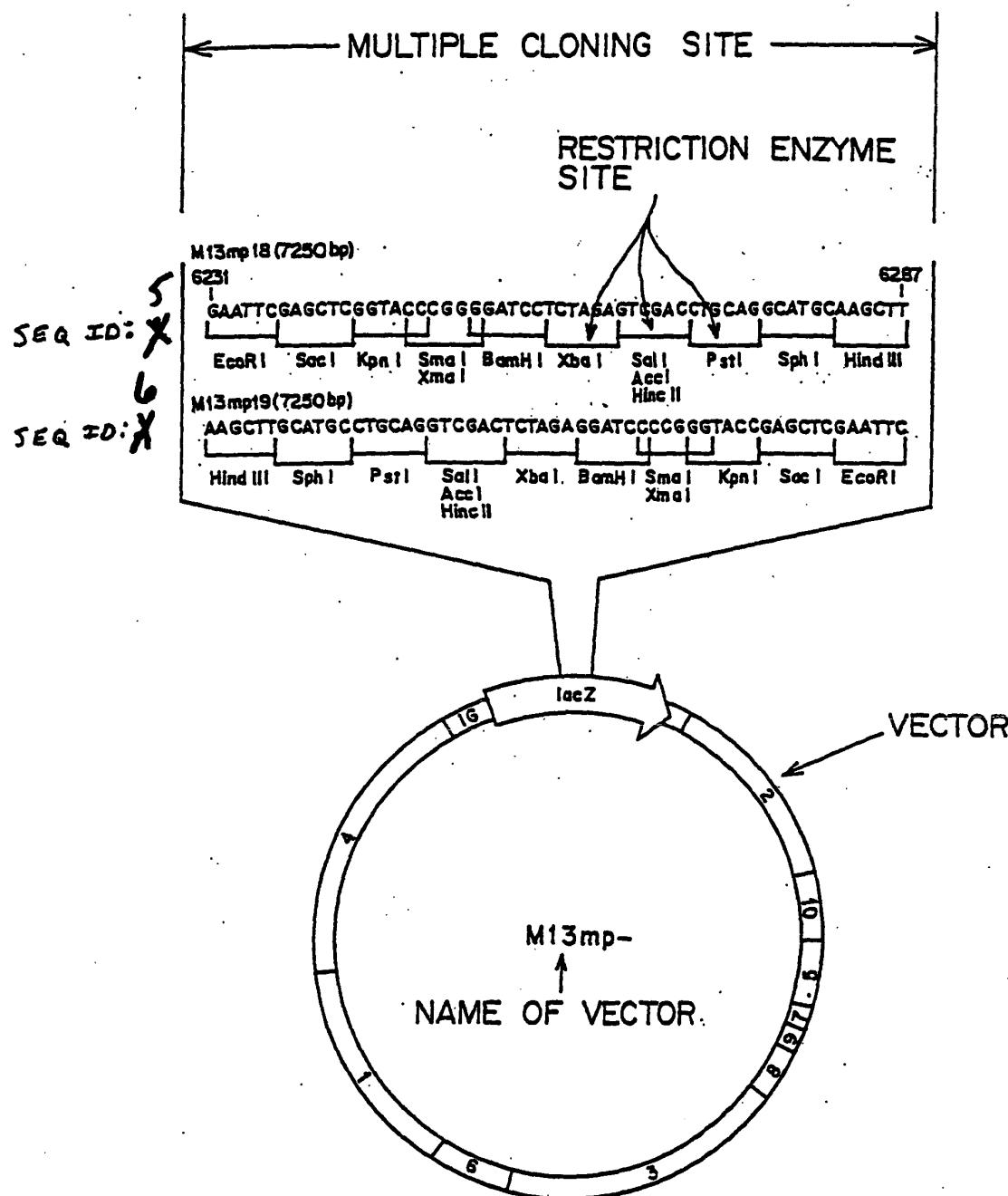


FIG. 3



TITLE METHOD AND APPARATUS FOR
AUTOMATICALLY REMOVING VECTOR UNIT IN
DNA BASE SEQUENCE

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE

SEQ ID: 87
32 (P) - TGCACTTGAACGCATGCT

ELECTROPHORESIS

UNLABELED ENZYME

RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL CLEAVING WITH A SPECIFIC ENZYME

SEQ ID: 19

SEQ ID: 19

SEQ ID: 19

SEQ ID: 20

32 P - TGC ACT TGAACGC

32 P - TGC ACT TGA

32 P - TGC ACT TG

32 P - TGC

TGC

CGCATGCT

ACGGATGCT

CTTGAACGGATGCT

THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4



TITLE METHOD AND APPARATUS FOR
AUTOMATICALLY REMOVING VECTOR UNIT
DNA BASE SEQUENCE
INVENTORS: Kensaku IMAI, et al.
SERIAL NO.: 09/785,269
DOCKET NO.: 826.1335C

VECTOR DB FORMAT

> ID

PUC18

>SEQ 10: X //

TCGCGCGCTTCGGTGTAGACGGTGAACACATGACATGAGCTCCCGAGACGGTCAGCTGGCTTAACATGCGGATCAGA
GCCGGGAGCAGACAAGCCCGTCAAGGGCGCTAGGGCGCTAGGGGGTGTGGGGGCTGGCTTAACATGCGGATCAGA
GCAGATTGTACTGAGAGTGCACCATATGCGGTGAAATACCGACAGATGCGTAAGGAGAAAATACCGCATCAGGCGC
ATTGCGCATTCAGGCTGCGCACTGTTGGAAAGGGCGATCGGTGGGGCTCTCGCTTAAACCGCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAGTGGGTAACGCCAGGGTTTCCAGTCAGGACGTTGAAAACGACGGCAGTGCAA
GCTTGATGCGCTGAGGTCAGCTAGAAGGATCCCGGGTACCGAGTCAGTCAATCATGGTATAGCTGTTCC
GTGTGAAATTGTATCCGCTCACAAATTCCACACAATACGAGCGGAGAACATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAAGCTAACTCACATTAAATTGCGTTCGCTCACTGCCCCGTTTCAGTCGGGAAACCTGCGTGCAGCTGCAATTAA
GAATCGGCAACCGCGGGGAGAGGGCGTTTGCCTTGGGCGCTTCCGCTTCCGCTCACTGACTCGCTGCGCTCG
GTCGTTCCGCTGCGGAGCGGTATCAGCTCACTCAAAGGCGTAATACGGTTATCCACAGAAATCAGGGATAACGAGG
AAAGAACATGTGAGCAAAGGCGAGCAAAGGCGAGGAACCGTAAAAGGCGCGTGTGGCGTTTCCATAGGCTCC
GCCCGGCTGAGGAGCATCACAAAATGACGCTCAAGTCAGGTTGGCGAAACCGACAGGACTATAAGATAACGAGGCG
TTTCCCGTGGAGCTCCCTGTCGCTCTCTGTCGACCCCTGCGCTTACCGGATACTGTCGCGCTTCTCCCTC
GGGAAGCGTGGCGCTTCTCAAAGCTACGCTGTAGGTATCTCAGTCGGTGTAGGCTGTCGCTCAGCTGGGCTGTG
TGCACGAACCCCCCGTTCAGCCCCACCGCTGCGCTTATCGGTAACTATCGTCTTGTAGTCCACCGGTAAGACAGAC
TTATGCCACTGGCAGCAGCCACTGGTAACAGGATTAAGCAGAGCGAGGTATGAGGGCTGTCAGAGTTCTGAGTG
GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTCGGAAAAAGAG
TTGGTAGCTCTTGATCCGGAAACAAACCCACCGCTGGTAGCGGGGGTTTTTTGTTGCAAGCAGCAGATTACGCGCAGA
AAAAAAGGATCTCAAGAAGATCTTGTCTTCTACGGGGTCTGAGCTCACTGGGAAACGAAAATCACGTTAAGGGAT
TTGGTCTAGAGATTATCAAAGGATCTTACCTAGATCTTTAAATTAAAAATGAAGTTAAATCAATCTAAAGTA
TATATGAGTAAACTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGCTATTCTGTTCA
TCCATAGTGCCTGACTCCCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGGCCAGTGTGCTGCAATGAT
ACCGCAGACCCACGCTACCGGCTCAGGATTATCAGCAATAAACAGCCAGCGGAAGGGCGAGCGCAGAAGTGGTC
CTGCAACTTATCGCCCTCATCCAGTCTATTAAATTGTCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTG
CGCAACGTTGCCATTGCTACAGGCATCGGGTGTAGCTCGTGTGGTATGGCTTCACTCAGCTCCGGTCCCA
ACGATCAAGGCGAGTTACATGATCCCCATGTTGCAAAAAAGGGTTAGCTCTCGGTCTCCGATCGTTGTCAGAA
GTAAGTTGCCGAGTGTATCACTCATGGTTATGGCAGCACTGCTAAATTCTTACTGTCATGCCATCCGTAAGTGC
TTTCTGACTGGTGTAGTACTCAACAAAGTCATTGAGAAATAGTGTATGCCGACCGAGTGTCTTGGCGCTC
AATACGGGATAATACCGGCCACATAGCAGAACTTTAAAGTGTCTCATTTGGAAAACGTTCTGGGGCGAAAACCT
CAAGGATCTTACCGCTGTGAGATCCAGTTCAGTGTAAACCACTCGCACCCAACTGATCTCAGCATCTTACTTC
ACCAAGCTTCTGGGTGAGCAAACAGGAAGGAAAATGCCCAAAAAAGGGATAAGGGCGACCGAAATGTGAAT
ACTCATACTCTCCTTTCAATTATTGAGCAATTACGGTTATTGTCATGAGGGATAACATATTGAGTGA
TTAGAAAAAAACAAATAGGGTCCGCGACATTCCCGAAAAGTGCACCTGACGCTAAGAAACCAATTATC
ATGACATTAACTATAAAAGGCGTATCACGAGGCCCTTCGTC

>MULTI
399..45

FIG. 9

METHOD AND APPARATUS FOR
AUTOMATICALLY REMOVING VECTOR UNIT IN
DNA BASE SEQUENCE
INVENTORS: Kensaku IMAI, et al.
SERIAL NO.: 09/785,269
DOCKET NO.: 826.1335C



(* INDICATES MULTIPLE CLONING SITE)

SEQ ID: 8 GTGCCAAGCTTGATGCCCTGCAGGTCGACTCTAGAGGATCCCCGGTACCGAGCTCGAAATTCTGTAAT

SEQ ID: 9 ¹² AAGCTT \Rightarrow HIND III

SEQ ID: 10 ¹² GCATGC \Rightarrow SPH I

SEQ ID: 11 ¹⁵ CTGCAG \Rightarrow PST I

SEQ ID: 12 GTCGAC \Rightarrow SAL I, ACC I, HINC II

SEQ ID: 13 ¹⁷ TCTAGA \Rightarrow XBA I

SEQ ID: 14 ¹⁹ GGATCC \Rightarrow BAMH I

SEQ ID: 15 ²⁰ CCCGGG \Rightarrow SMA I, XMA I

SEQ ID: 16 ²¹ GGTACC \Rightarrow KPN I

SEQ ID: 17 ²² GAGCTC \Rightarrow SAC I

SEQ ID: 18 ²³ GAATTTC \Rightarrow BCG I

FIG. 10

WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE
XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS
SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS
SPECIFIED ON OBJECT DNA 3' SIDE

(**** INDICATES RESIDUAL MULTIPLE CLONING SITE
(+ + + + INDICATES AN OBJECT DNA FRAGMENT

(SEQUENCE
ID NO. 4)

GTGCCAAGCTT
AAGCTT
↑

5' SIDE RETRIEVAL KEY
(IN THIS EXAMPLE,
HIND III SITE)

***** (SEQUENCE ID NO. 23)
TCTAGAGGATCCCCGGGTACCGAGCTCGAATTCTGAAT
TCTAGA
↑

9' SIDE RETRIEVAL KEY
(IN THIS EXAMPLE, XBA I SITE)

FIG. 17